

The calculated manipulation of DNA is a relatively recent accomplishment of science, it was only in the 1970s that scientists began experimenting with recombinant DNA. They found that when the recombinant DNA molecule was placed in new cells, these cells would synthesize the proteins encoded for by the newly introduced genes. In this plate we will study how this occurs.

This plate continues to describe the process of genetic engineering, or recombinant DNA technology. A segment of DNA from a foreign cell is first inserted into a fragment of DNA, and the resulting recombinant DNA is then placed into fresh cells, where the genes are expressed.

The process of genetic engineering caused a revolution in biotechnology. Genetic engineering techniques are currently being used to study processes such as gene regulation, to develop and produce products such as human hormones, to create disease-resistant plants, and to diagnose and treat genetic disorders. In this plate we study how genetic engineering can be used to synthesize large quantities of human insulin from bacterial cells. Insulin is a pancreatic hormone in which diabetics are deficient; it facilitates the uptake of glucose by cells.

We begin the genetic engineering process with a bacterium and a human cell. The bacterium (A) is shown at the top left. A light color should be used to shade it. The bacterial chromosome (B) appears as a highly coiled loop of DNA. Also necessary for the process of genetic engineering is a tiny loop of DNA called a plasmid (C). The plasmid consists of approximately twenty genes and exists freely in the cytoplasm; a bacterium normally contains numerous plasmids.

On the right you can see the human cell (D), which should be colored lightly. The nucleus (E) in this diagram contains only one human chromosome (F) for simplicity. This particular chromosome is the site of the insulin-encoding genes.

We have established the two sources of DNA for genetic engineering. The bacterium supplies plasmid DNA while the human cell supplies chromosomal DNA.

The next step in this process is to produce recombinant DNA. First, a restriction enzyme (G₁) is used to open the plasmid at the left. The enzyme acts at a restricted point (explained in the previous plate) and creates an opened plasmid (J). At the points at which the enzyme has spliced the plasmid, the DNA has sticky ends (I). You should color the arrow that points to the sticky ends. Next, the same restriction enzyme (G₁) is used to splice the human chromosome, and the result of this splicing is that the insulin genes (H) are isolated. These gene fragments also have sticky ends (I), and these are indicated by the arrow.

Now the recombinant DNA molecule is created. Both the opened plasmid (J) and the insulin fragment bear DNA that has sticky ends, and these sticky ends are complementary. This means that when they are brought together, they overlap, and then ligase (G₂) is used to seal the ends together. This ligase creates two hydrogen bonds between adenine and thymine bases and three between guanine and cytosine bases. The result is a recombinant plasmid, also known as a chimera (K).

The genetic engineering process has now progressed to the point where the insulin gene has been inserted into a bacterial plasmid. The recombinant plasmid (chimera) will now be inserted into new cells.

Recombined plasmids can be inserted into new bacterial cells by alternately heating and cooling the cells. When this process is completed, the recombined plasmid (K) is inside a new bacterium (L). Now the bacterial cell is allowed to grow and multiply by placing it in an enriched environment. As these metabolizing bacteria (M) grow and increase in number they carry on their normal metabolic activities. All bacteria have the insulin genes in their new, recombined plasmids, and these genes now operate and encode the protein insulin. Before long, the bacterium will begin producing insulin (N). We therefore have the unusual situation of a bacterial cell that produces human insulin!

For many decades insulin was isolated from the pancreases of animals, and diabetics depended on this insulin to allay the symptoms of their disease. It is now possible to obtain large amounts of insulin from metabolizing bacteria. This is one example of where genetic engineering has made a substantial impact on human welfare.

4-15: Genetic Engineering

a. When recombinant DNA was placed in new cells, these cells would synthesize:

b. What are some ways genetic engineering techniques are being used?

c. What does insulin facilitate?

d. Tiny loop of DNA in a bacterium:

e. How many genes does a plasmid contain?

f. Does a bacterium usually contain more than one plasmid?

g. What is used to open the plasmid?

h. Are the ends sticky?

i. What does the restriction enzyme isolate from the human chromosome?

j. Are the ends sticky?

k. The plasmid & insulin DNA are sealed together by:

l. Another name for a recombined plasmid:

m. Where does the recombined plasmid go?

n. As they grow & multiply, what will they also produce?

